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Cu EXAFS of CO Dehydrogenase/Acetyl-CoA Synthase

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Beamline(s): X18B

Introduction: CODH/ACS plays a central role in the Wood-Ljungdahl pathway of autotrophic CO2 fixation [1]. CODH catalyzes the two-electron reduction of CO_2 to CO and ACS catalyzes acetyl-CoA synthesis from CO, the methyl-donor and CoA. The active site for the ACS activity is called cluster A. Recent crystal structure reveals that cluster A contains 1 Ni, 1 Cu, and 4Fe ions [2]. The discovery of Cu at the ACS active site was surprising since until recently [4], Cu was not known to be a component of CODH/ACS. In order to confirm the crystallography result and study the functional roles of Cu, we measured the Cu EXAFS of CODH/ACS.

Methods and Materials: *Moorella thermoacetica* was grown with glucose as the carbon source at 55 ^{o}C. CODH/ACS was purified and loaded into EXAFS sample holder under strictly anaerobic conditions anaerobic chamber (Oxygen tension below 1ppm).

Results: Figure 1 shows the Cu K-edge spectrum of CODH/ACS. The inflection point energy of 8983 eV is similar to Cu(I) edge reported previously [3].The shape of the edge clearly rules out that 2-coordinate Cu(I). The shape of the edge is intermediate between the K-edge reported for other Cu(I)S3 and Cu(I)S4 species [3]. Cu EXAFS spectrum (Figure 2) was first fit by 3 S at 2.25 Å. Adding one more S increases the Debye-Waller factor to an unreasonably high value. There is another dramatic feature at ~2.6 Å in the FT (phase shifted) spectrum, which could be fit by a Cu-metal (Ni) interaction at 2.65 Å. The Cu-S and Cu-Ni distances obtained from the EXAFS fit are consistent with the crystal structure [2].

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References:

[1]. S. W. Ragsdale, and M. Kumar, “Nickel-Containing Carbon Monoxide Dehydrogenase/Acetyl-Coa Synthase” *Chem. Rev.* **96,** 2515-2539 (1996).

[2]. T. I.Doukov, T.Iverson, J.Seravalli, S. W. Ragsdale, and C. L. Drennan, “A Ni-Fe-Cu Center in a Bifunctional Carbon Monoxide Dehydrogenase/Acetyl-CoA Synthase” *Science* **298,** 567-572 (2002).

[3]. L.S. Kau, D.J. Spira-Solomon, J.E. Pnner-Hahn, K.O. Hodgson and E.I. Solomon, “X-ray Absorption Edge Determination of the Oxidation State and Coordination Number of Copper: Application to the Type 3 Site in *Rhus vernicifera* Laccase and Its Reaction with Oxygen” *J.Am.Chem.Soc.* **109,** 6433-6442 (1987).

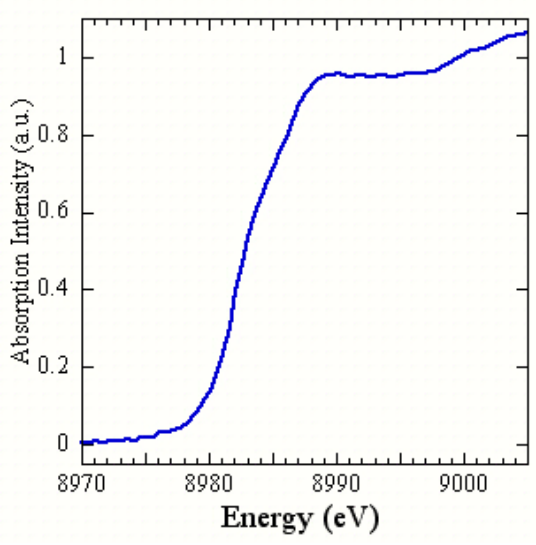

Figure 1. Cu K-edge spectrum of CODH/ACS.

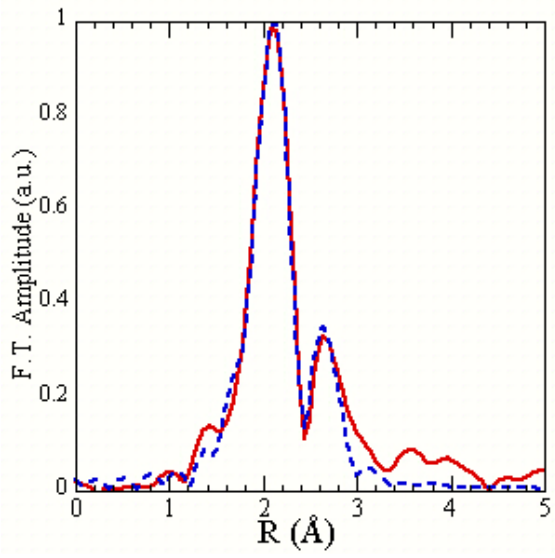

Figure 2. Cu F.T. EXAFS (solid) and best fit (dash).